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Genetic diversity of *Taenia hydatigena* in the northern part of the West Bank, Palestine as determined by mitochondrial DNA sequences

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Abstract

Cysticercus tenuicollis is the metacestode of canine tapeworm *Taenia hydatigena*, which has been reported in domestic and wild ruminants and is causing veterinary and economic losses in the meat industry. This study was conducted to determine the sequence variation in the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene in 20 isolates of *T. hydatigena* metacestodes (cysticercus tenuicollis) collected from northern West Bank in Palestine. Nine haplotypes were detected, with one prevailing (55%). The total haplotype diversity (0.705) and the total nucleotide diversity (0.0045) displayed low genetic diversity among our isolates. Haplotype analysis showed a star-shaped network with a centrally positioned common haplotype. The Tajima's D, and Fu and Li's statistics in cysticercus tenuicollis population of this region showed a negative value, indicating deviations from neutrality and both suggested recent population expansion for the population. The findings of this study would greatly help to implement control and preventive measures for *T. hydatigena* larvae infection in Palestine.

Keywords

Taenia hydatigena, genetic variation, cysticercus tenuicollis, cytochrome c oxidase subunit I, Palestine

Introduction

Cysticercus tenuicollis is the metacestode of canine tapeworm *Taenia hydatigena*, which has been reported in domestic and wild ruminants (Soulsby 1982; Murrell and Dorny 2005). In recent years, it has become clear that greater priority should be given to cysticercus tenuicollis because of its veterinary and economic losses in the meat industry, especially in undeveloped countries (Nourani *et al*. 2010; Samuel *et al*. 2010; Scala *et al*. 2015). Palestine depends largely on its agriculture and veterinary resources. It is important that measures should be taken to reduce economic losses caused by avoidable environmental infection.

The prevalence of cysticercus tenuicollis among domestic ruminants and its negative effect on the meat industry and the economy in Palestine have not been determined. Genetic diversity of *T. hydatigena* has never been characterized in the region especially in Palestine. This study was designed to investigate *T. hydatigena* larvae infection, the prevalence of cysticercus tenuicollis among sheep in the northern part of the West Bank (Palestine), and most importantly to determine the genetic diversity of *T. hydatigena* based on mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene.

The outcome of study should provide important information about *T. hydatigena* strains distribution, infection and genetics. This will help Palestine and the region to develop tools and measures which are more effective for eradication of *T. hydatigena* larvae infection and to improve national economy.

Materials and Methods

Samples collection

A total of 1489 sheep were investigated in this study for the presence of cysticercus tenuicollis. During the period of April to June 2014, all 1489 sheep slaughtered at the municipal abattoir of Nablus, Northern part of the West Bank,

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Palestine were visually inspected for the presence of cysticercus tenuicollis. The slaughtered sheep were both males and females, originated from three different parts in the northern districts of the West Bank, Palestine. To evaluate the effect of age, sheep were classified into two groups: young (less than 1 year) and adult (more than 1 year). Animals suspected with the presence of cysticercus tenuicollis were identified and information were recorded especially for age, geographic location and gender. Cysts were collected from each abnormal animal, followed by rinsing in saline and then stored at –20°C for further studies.

PCR Amplification, purification, and sequencing

Total DNA was extracted as described by Yamasaki *et al*. (2005), with slight modifications. Briefly, part of each cyst was cut into small pieces and were lysed in 50 to 60 µl of 0.02 N sodium hydroxide containing 10 to 20 µl of 10 mg/ml proteinase K at 90°C for 30 min. Following chloroform extraction and purification, all DNA samples were stored at –20°C. The *cox1*gene was amplified using primers 5′-TTT TTT GGG CAT CCT GAG GTT TAT (forward) and 5′- TAA AGA AAG AAC ATA ATG AAA ATG (reverse). The PCR was performed with a Ready Mix PCR kit (Sigma-Aldrich Co, St Louis, MO). Reaction mixtures contained 2 µl template DNA, 12.5 µl master mix with, 2.5 µl primer mix (1 µM for each primer) and ribonuclease-free water to a final volume of 25 µl. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf). Thermal cycling was performed with initial denaturation for 5 min at 95°C followed by 35 cycles of 1 min at 95°C, 1 min at 50°C and 1 min at 72°C, and a final extension of 5 min at 72°C. PCR products were sequenced and the sequences were assessed with the following GenBank accession numbers: KM032284- KM0322303.

Table I. Prevalence of cysticercus tenuicollis in relation to sex and age among sheep examined at the municipal abattoir of Nablus, Palestine

Variable		No. of animals examined	No. (%) infected	*p* value
Sex	Male	928	25 (2.7)	NS
	Female	561	7 (1.2)	
Age	Young	1343	26 (1.9)	NS
	Adult	146	6 (4.1)	
Total number		1489	32 (2.2)	

NS, not statistically significant ($p > 0.05$).

Data analysis

Multiple alignments of the *cox1* nucleotide sequences were performed in ClustalW. The phylogenetic analysis was constructed using the neighbor-joining (NJ) method in CLC Main Workbench software (version 7.0.3, 2014, CLC bio, Aarhus, Denmark). The population diversity indices such as numbers of haplotype (h), haplotype diversity (Hd), nucleotide diversity (π), and the neutrality indices including (Tajima's D, and Fu and Li's statistics) were calculated using DnaSP 5.10 (Librado and Rozas 2009). Popart-1.7 (Bandelt *et al*. 1999) was used to analyze the MJ-network of haplotypes.

Results

Out of 1468 sheep slaughtered at the municipal abattoir of Nablus, Northern part of the West Bank, Palestine, 32 sheep (2.15%) were infected with cysticercus tenuicollis cysts. Most cysts were found to be in the liver. Out of 32 animals with cysts, a total of 30 (93.8%) have originated from the Liver. The data also revealed no effect of age and sex on the preva-

Fig. 1. Phylogenetic relationship of cysticercus tenuicollis samples using *cox1* gene computed by neighbor joining (NJ). *E. granulosus* HM626406 was used as an outgroup strain. The scale bar represents the estimated number of nucleotide substitutions per nucleotide site

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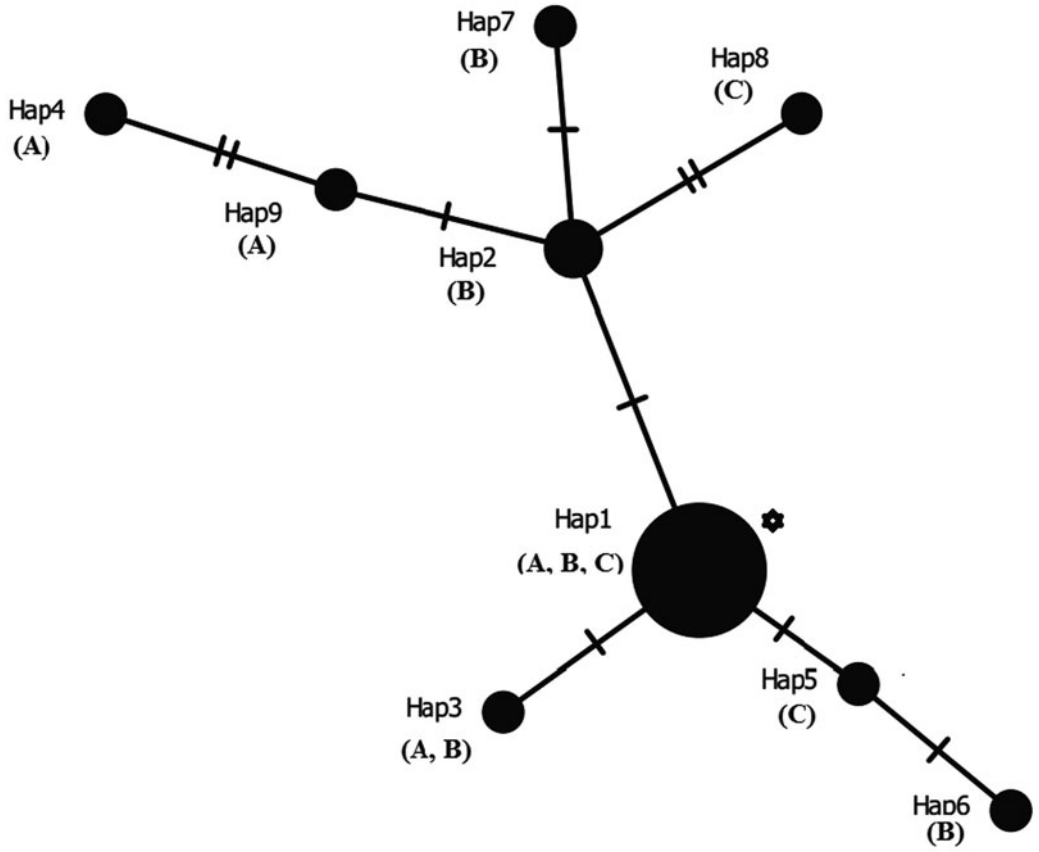

Fig. 2. Median-joining network of *cox1* gene of the haplotypes of cysticercus tenuicollis isolates. Each haplotype is represented by a circle. The asterisk (*) denotes the founder haplotype. The size of circle is relative to haplotype frequency. Bars indicate the number of nucleotide substitutions. The geographical distribution of each haplotype was presented in brackets (3 different districts: A, B and C)

lence of the disease among the examined sheep (p values were 0.06288 and 0.08914, respectively) (Table I).

DNA extraction was performed, and the *cox1* gene was amplified in all of the samples (n = 32) with amplicon size of 444-bp. To understand the genetic diversity of this parasite in Palestine, *cox1* nucleotide sequences were analyzed in 20 cysticercus tenuicollis isolates. Nine haplotypes were detected, with the main haplotype being Hap1 (11 out of 20 isolates, 55%) (Fig. 1). The haplotype diversity was 0.705, while the nucleotide diversity was 0.0045. The MJ networks constructed

Table II. Nucleotide variation positions of *cox1* gene between the nine haplotypes found in this study. Positions at which no variation was found are marked with dot marks, whereas a letter indicates a variant nucleotide at this position. Singleton substitutions have a white background and parsimoniously informative sites are shaded in grey

Haplotype	No. of isolates	Position of nucleotide change									
		6	51	72	102	141	207	213	219	231	264
1	11	T	A	T	T	T	T	G	T	C	A
2	2	.	.	.	.	.	.	A	.	.	.
3	1	C	.	.	.	.	.	.	.	.	.
4	1	.	.	C	.	.	.	A	C	.	G
5	1	.	G	.	.	.	.	.	.	.	.
6	1	.	G	.	.	C	.	.	.	.	.
7	1	.	.	.	G	.	.	A	.	.	.
8	1	.	.	.	.	.	C	A	.	T	.
9	1	.	.	.	.	.	.	A	C	.	.

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from haplotypes of *cox1* sequences showed a star-like expansion with a major central haplotype (Hap1). The numbers of mutational steps between the major haplotype and the others ranged from one to four, and the frequency of the major haplotype was 55% in the population (Fig. 2). Overall, there were 10 point mutations between the major core haplotype and the other haplotypes, including 7 singleton variable sites (SP) at positions 6, 72, 102, 141, 207, 231, 264 and 3 parsimony informative sites (PIP) at positions 51, 213 and 219 (Table II). The neutrality test showed that Tajima's $D = -1.60988$ ($0.1 > P > 0.05$), Fu and Li's $D = -1.91648$ ($P > 0.1$), Fu and Li's $F = -2.11821$ ($P > 0.1$) were not significantly different.

Discussion

This is the first study conducted to obtain a snapshot on the prevalence of cysticercus tenuicollis cyst in Palestinian sheep and to study the genetic variation and population structure of cysticercus tenuicollis circulating in Palestine. The overall prevalence of cysticercus tenuicollis cyst was 2.15% and the liver was the most infected organ. The results of this study indicated that cysticercus tenuicollis has become a serious problem in Palestine, but remains relatively low compared to those that have been reported in many other countries including nearby countries in the Middle East area (Dajani and Khalaf 1981; Hasslinger and Weber-Werringhen 1988; Senlik, 2008; Sultan *et al*. 2010; Nimbalkar *et al*. 2011; Oryan *et al*. 2012; Mekuria *et al*. 2013; Scala *et al*. 2015). This low prevalence is probably due, in part, to the sheep flock traditional smallholder management system prevailing in Palestine.

The *cox1* gene of taeniid cestodes has already been shown to be a promising candidate for the classification of intra- and interspecific variants (Bowles *et al*. 1992). Therefore, 444-bp *cox1* gene sequences of 20 isolates were used in this study. Phylogenetic analysis of these sequences indicated the existence of nine haplotypes with a major haplotype being Hap1. The total haplotype diversity (0.705) and the total nucleotide diversity (0.0045) were low, suggesting that Palestinian *T. hydatigena* metacestode haplotypes were not genetically differentiated. This is also evident from the NJ network analysis, which revealed a star-like expansion of the haplotypes from a main founder haplotype (Hap1) with mostly 1–4 mutational steps. Moreover, no geographical clustering was observed from the NJ tree analysis

The genetic variation within the *cox1* observed in this study was lower than those reported in other countries (Rostami *et al*. 2015; Boufana *et al*. 2015a). This could be due to the lower prevalence and transmission rate of cysticercus tenuicollis, smaller area of Palestine as well as the prevailing management system here.

Additionally, self-fertilization in hermaphrodites may maintain mediate gene flow leading to intraspecific phenotypic uniformity in taeniid populations (Nakao *et al*. 2003; Boufana *et al*. 2015b). This is consistent with the low haplotype variation observed in the current study for *T. hydatigena* metacestodes derived from sheep hosts.

Neutrality tests, such as Tajima's *D*, Fu and Li 's D, and Fu and Li's F tests have been developed to test selective neutrality of nucleotide variability and they are used to find out the population expansion (Ramos-Onsins and Rozas 2002). In this study, the overall negative values of both neutrality tests detected among *cox1* sequences for Palestinian *T. hydatigena* metacestode haplotypes indicated an excess of low frequency polymorphisms in population, which may imply deviations from neutrality and both suggested recent cysticercus tenuicollis population expansion after the introduction of founder haplotype.

In conclusion, the results presented in this study showed that the existing genetic status of Palestinian sheep *T. hydatigena* metacestode population keep a low genetic diversity based on mitochondrial region. This data provided useful information for future studies focusing on improving diagnosis and prevention methods and developing robust control strategies. Finally, to evaluate more in-depth the molecular ecology and population genetics of cysticercus tenuicollis, additional studies should be performed using a larger number of cysticercus tenuicollis isolates from Palestine and other geographical regions. Additionally, investigating more informative genes beside the *cox1* is also needed.

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